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L6: Entry 1 of 3

File: USPT

May 14, 2002

DOCUMENT-IDENTIFIER: US 6387626 B1

TITLE: Covalent attachment of unmodified nucleic acids to silanized solid phase surfaces

Detailed Description Text (12):

In the most preferred embodiment, the invention provides for methods of covalent attachment of unmodified oligonucleotides onto mercapto-silanized surface or epoxy-silanized surfaces with high density and high stability. The ease of preparation of unmodified oligonucleotides coupled with stable ether (epoxy) or thio-ether (mercapto) linkage attachments renders this method the most cost effective, with little or no variation in terms of the quality of oligonucleotides, stability of attachment linkage and consistency in large scale batch to batch manufactures. Additionally, the hydrophobic property of silane surfaces also allows simultaneous patterning of multiple DNA probes in a high density and in a variety of array formats. Furthermore, a DNA array that is stable to high salt and denaturing conditions such as DMF, urea and elevated temperatures, has wide uses in miniaturized biotechniques such as genetic testing, sequencing by hybridization and combinatory selection of DNA binding molecules.

Detailed Description Text (45):

Using the method described in the present patent application, oligonucleotide primers can be immobilized on solid phases like polystyrene or glass, hybridized to PCR-derived, single-stranded templates, and subjected to enzymatic extension at their 3'-ends by a single, labeled ddNTP. The nature of the incorporated ddNTP is determined by the nucleotide that is located in the opposite strand (the polymorphic nucleotide). This assay can be conveniently carried out both in polystyrene ELISA plates, or on glass slides.

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=> s oligonucleotide(10a)immobiliz##(10a)glass(10a)(urea or acetamide)
             0 OLIGONUCLEOTIDE(10A) IMMOBILIZ##(10A) GLASS(10A)(UREA OR ACETAMI
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     Oligonucleotide probe immobilization on nylon membranes
ΤI
     Kawasaki, Ernest S.; Levenson, Corey H.; Will, Stephen G.; Zhang, Yong
IN
     Hoffmann-La Roche, F., und Co. A.-G., Switz.
PA
SO
     Eur. Pat. Appl., 17 pp.
     CODEN: EPXXDW
DT
     Patent
LA
     English
FAN.CNT 1
     PATENT NO.
                     KIND DATE
                                           APPLICATION NO. DATE
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                A1 19921104
                                         EP 1992-106603
     (EP 511559)
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     R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE
                     A1 19921105 AU 1992-15062
     AU 9215062
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CA 2067426

19930920

AA 19921031

ZA 1992-2950

CA 1992-2067426 19920428

19920423

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                              19930702
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PRAI US 1991-694226
                              19910430
     Oligonucleotide probes are immobilized on nylon membranes with a high d.
     of anionic carboxyl groups via an amide bond. A method for attachment of the probes to the membrane is also disclosed. The single (5'-end)
     attachment of the oligonucleotides to the membrane surface leaves the
     probe free to interact with complementary sequences, thus increasing the
     hybridization efficiency relative to probes attached by methods in
     which heat or UV light is used for immobilization. The simplicity and
     reproducibility of this method and the sensitivity attained when using the
     reagents produced by the method are ideal for application of the method
     and reagents to the diagnosis of infectious and genetic diseases, the
     anal. of mutations in neoplasias, HLA typing, and other areas.
     Immobilized probes for detection of a cystic fibrosis mutant sequence and
     a RAS mutant sequence (including 3 RAS probes, differing by only 1 base
     and representing the possible sequence changes in codon 12 of N-RAS,
     immobilized on 1 dot) were tested.
AΒ
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     of anionic carboxyl groups via an amide bond. A method for attachment of the probes to the membrane is also disclosed. The single (5'-end)
     attachment of the oligonucleotides to the membrane surface leaves the
     probe free to interact with complementary sequences, thus increasing the
     hybridization efficiency relative to probes attached by methods in
     which heat or UV light is used for immobilization. The simplicity and
     reproducibility of this method and the sensitivity attained when using the
     reagents produced by the method are ideal for application of the method
     and reagents to the diagnosis of infectious and genetic diseases, the
     anal. of mutations in neoplasias, HLA typing, and other areas. Immobilized probes for detection of a cystic fibrosis mutant sequence and
     a RAS mutant sequence (including 3 RAS probes, differing by only 1 base
     and representing the possible sequence changes in codon 12 of N-RAS,
     immobilized on 1 dot) were tested.
ST
     oligonucleotide hybridization probe immobilization membrane;
     cystic fibrosis mutation probe immobilization; RAS oncogene mutation probe
     immobilization
IT
     Carboxyl group
        (anionic, nylon membrane with high d. of, oligonucleotide
        hybridization probes immobilization on)
ΙT
     Cystic fibrosis
        (gene for, mutation in, hybridization probes for detection
        of, immobilization on nylon membranes of)
IT
     Mutation
        (in cystic fibrosis or c-ras genes, hybridization probes for
        detection of, immobilization on nylon membranes of)
IT
     Polyamides, uses
     RL: USES (Uses)
        (membrane of, oligonucleotide hybridization probes
        immobilization on)
ΙT
     Membranes
        (nylon, oligonucleotide hybridization probes immobilization
        on)
TT
     Amides, uses
     RL: USES (Uses)
        (oligonucleotide hybridization probes immobilization on nylon
        membrane by)
IΤ
     Nucleic acid hybridization
        (oligonucleotide probes immobilized on nylon membrane for)
ΙT
     Nucleotides, polymers
     RL: BIOL (Biological study)
        (oligo-, immobilization on nylon membrane of, for hybridization
```

assay)

Recombination, genetic

IT

(translocation, hybridization probes for detection of, immobilization on nylon membranes of) ΙT Gene, animal RL: BIOL (Biological study) (c-ras, mutation in, hybridization probes for detection of, immobilization on nylon membranes of) 57-13-6D, Urea, O-acyl derivs. IΤ RL: FORM (Formation, nonpreparative) (formation of, in oligonucleotide probe immobilization on nylon membrane) 147014-43-5, Biodyne C ΙT RL: USES (Uses) (membrane of, oligonucleotide hybridization probes immobilization on) 9035-51-2, Cytochrome P450, analysis ITRL: ANST (Analytical study) (nucleics acids encoding, hybridization probes for detection of, immobilization on nylon membranes of) 147178-33-4D, peroxidase conjugates 147178-34-5D, peroxidase conjugates IT 147178-35-6D, peroxidase conjugates RL: USES (Uses) (oligonucleotide probe for RAS sequence detection immobilizated on nylon membrane hybridization to)

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